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(57) Abstract

Solid vaccine compositions comprise an antigenic substance, a saponin and a polycationic adjuvant such as DEAE-dextran. The antigenic substance gives rise to antibodies either for the purpose of fighting infections or for other purposes: for example, antibodies against GnRH can modulate fertility. The combination of a saponin and a polycationic adjuvant gives the vaccine improved longevity and enables it to be used as an implant.

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VACCINES

This invention relates to vaccines.

Vaccines have classically been used in the prevention of disease. An antigen having antigenic characteristics of a disease-causing entity (such as a microbe or toxin) is parenterally administered to man or another animal, and the animal's immune system is stimulated to produce antibodies which will react both with the antigen administered and the disease-causing agent itself.

More recently, vaccines have also been used for other purposes, particularly in the modulation of hormonal activity. Antibodies generated against a hormone antigen may cross react with endogenous hormone in the animal's body. A primary (but not exclusive) application of this new vaccine technology is the production of vaccines for fertility control.

The antigenicity of many potential antigens is frequently enhanced by the co-application of antigens with immunoadjuvants, which may be regarded as substances which, while not necessarily being antigenic themselves, potentiate or enhance an animal's immune response to the challenging antigen.

A wide range of adjuvants is known. Examples include Freund's complete and incomplete adjuvants (FCA and FIA), saponins, aluminium compounds, including aluminium phosphate and aluminium hydroxide (particularly in the form known as alhydrogel),

polycationic electrolytes, polyanionic electrolytes, muramyl dipeptide and Adjuvant 65, which contains highly refined peanut oil and chemically pure mannide monooleate and aluminium monostearate as emulsifier and stabiliser respectively.

Even with the availability of the above and many other adjuvants, it is sometimes difficult to formulate vaccines for inducing antibodies against particular antigens. Gonadotrophin releasing hormone (GnRH, otherwise known as luteinising hormone releasing hormone (LHRH) is a case in point.

It is commercially desirable to formulate a GnRH vaccine for veterinary use, particularly but not exclusively for domestic livestock. An antigen GnRH preparation is useful as a fertility regulating or an immunological neutering vaccine in male (for immunocastration) and female (for immunospaying) animals. It is indicative of the difficulties of formulating a GnRH vaccine that the neutering properties of GnRH have been known since 1972, but it is only now that vaccines based on GnRH are beginning to emerge commercially [Hoskinson et al, Aust. J. Biotech, 4, 166-170(1990)]. The utility of a GnRH vaccine is demonstrated by the experiences of Australian stock farmers. In extensively grazed cattle raised for beef, up to 80% of the cull cows can become pregnant, thereby causing the farmer considerable economic loss at slaughter because the carcase value is downgraded.

GnRH can be formulated as a vaccine with Freund's complete adjuvant (FCA), which comprises a suspension of heat-killed M. tuberculosis mycobacteria in mineral oil containing a surfactant. Although FCA is recognised as a powerful adjuvant, it has not found wide application outside the laboratory because of the adverse tissue reaction it provokes in recipient animals. In fact, FCA is banned from veterinary use.

A different approach to the problem is disclosed in WO-A-8706129, which suggests the use of an implant containing microencapsulated immunogens of GnRH (or another antigen) within a biodegradable polymer. The level of development of this technology as a practical matter, is still unclear; however, no commercial product based on the technology appears yet to have been launched.

The only GnRH vaccine on the market is a two-shot mineral oil based emulsion vaccine in accordance with the teaching of WO-A-8801177 [Hoskinson et al, Aust J. Biotech, 4 166-170 (1990)]. Although excellent results can be obtained by the use of such a vaccine, it would be desirable to eliminate the necessity of having oil present, and it would also be desirable to improve the longevity of action of the vaccine so that two shots were not required. The problem with having the mineral oil present, is that it can cause localised irritation at the site of injection or implantation, leading among other undesirable effects, to the formation of sterile abscesses and granulomas; further, it is generally desirable to avoid the use of petrochemical-derived

materials in preparations administered to animals, particularly parenterally.

The problem with a two-shot vaccine is more of a practical one for the farmer. The farmer will want to muster his livestock once a year in order to tag the herd and also for other veterinary purposes. The vaccine can therefore be conveniently administered at the mustering. However, if a second muster is needed several weeks later for a second, booster vaccination, this represents a considerable expenditure of effort purely for vaccination purposes, as there is otherwise no need for the second muster. In pastoral regions where ovine footrot is a problem, there is a need for two or more booster vaccinations to maintain high antibody levels in the sheep during the critical season. Longevity of action is therefore a desirable goal for a vaccine in order to avoid the unnecessary handling of animals.

It can be seen that there is a need for a vaccine which at least partially solves one or both of the two problems discussed above. Furthermore, it would be preferred if the action of the vaccine was reversible, particularly for a fertility-regulating vaccine such as one based on GnRH, so as to widen the potential market for the vaccine, for example to include horses. Further, it would be preferred if an effective vaccine could be formulated in solid form, which resulted in minimal tissue reaction at the implantation site and which conferred user safety by minimising the possibility of a farmer injecting himself with the

formulation and was able to provide improved shelf life stability.

According to a first aspect of the present invention, there is provided a solid vaccine composition comprising an antigenic substance capable of inducing the generation of antibodies on parenteral administration to an animal, a saponin and a polycationic adjuvant.

Although saponin and polycationic compounds have individually been used as adjuvants in the past, as have many other adjuvants, the art does not seem to have realised that this particular combination of adjuvants, when formulated as a solid, has particularly beneficial properties when used in a vaccine in accordance with this invention.

In the art, Solyom (Dev. Biol. Stand 34 169-178 (1977)) has separately evaluated DEAE-dextran (a polycationic adjuvant) and saponin in foot and mouth disease vaccines. Mitev et al (Vet. Med. Nauki. 12 16-22 (1975)) teaches that vaccines containing DEAE-dextran are generally inferior to oil-based vaccines; it is also suggested that saponin is a better sole adjuvant that DEAE-dextran. Gorskii (Uchenye Zap. Kazans. Vet. Inst. 122 48-49 (1976)) takes the opposite view to Mitev et al and teaches that DEAE-dextran is a superior adjuvant to saponin for foot and mouth disease virus. The efficacy of saponin, DEAE-dextran and aluminium hydroxide in a foot and mouth disease vaccine have also been evaluated in pig trials; here, DEAE-dextran performed better than $Al(OH)_3$ or saponin (Sellers and

Herniman Brit. Vet. J. 30 440-445 (1974)). The short lived nature of the immune response elicited to foot and mouth disease by DEAE-dextran or saponin has been described by Anderson et al (Res. in Vet. Sci. 12 351-357 (1971)). In contrast, this group demonstrate that oil-based emulsion adjuvants have longevity. The superior efficacy of Freund's adjuvant to others such as DEAE-dextran is described by Beh and Lascelles (Immunology 54 487-495 (1985)). Indeed, these authors state that no interactions between the different classes of adjuvant examined is observed. WO-A-8801177 teaches synergy between an oil adjuvant and a polycationic adjuvant; although this formulation is efficacious with GnRH and exhibits longevity, it relies on the presence of an oil-based emulsion; and the present invention avoids the use of oil. This type of synergy (where the immune response exceeds the sum of the immune responses of the individual components) is also observed by using dextran sulphate (a polyanionic adjuvant) in conjunction with saponin, Vanselow et al (Vet. Rec. 117 37-43 (1985)). WO 88/07547 teaches that the combination of DEAE-dextran and saponin in solution is useful at eliciting antibody when mixed with antigen; however it is known that such combinations, or the use of these adjuvants singly in solution, results in a short-lived immune response of little or no practical veterinary value. In contrast, the formulation of these adjuvants into a solid implant vaccine by the particular methods described here provides the basis for veterinary vaccines with longevity.

In a vaccine in accordance with the present invention, the antigenic substance may give rise to antibodies against a disease-causing agent, or against an agent (such as a hormone) which does not normally give rise to a disease. The disease causing agent may be a structural component or toxin of a virus, bacterium or other microbe. Examples of virally-caused diseases which may be controlled by means of the present invention include foot and mouth disease (FMD), infectious bursal disease (IBD), Newcastle disease, rabies, egg drop syndrome virus (EDS_{76}) disease in poultry, calcivirus, rhinotracheitis in cattle, bovine ephemeral fever (BEF) and respiratory virus, among others.

Examples of bacterially-caused diseases include botulism, clostridial infections, foot rot (for a vaccine against which the antigenic substance may comprise Bacterioides nodusus recombinant pili), Caseous Lymphadenitis CLA in sheep caused by Corynebacterium pseudotuberculosis toxin, among others. Other microbial, such as fungal or protozoal, infections may also be controlled by means of the present invention.

Of the vaccines in accordance with this invention which caused the generation of antibodies against non-disease-causing agents, a vaccine against GnRH is one of the most preferred. Vaccines against other peptide hormones (for example growth hormone) are also commercially significant as are vaccines against certain non-peptide hormones, for example steroid hormones.

The antigenic substance may consist of the entity against which antibodies are to be raised. This may frequently be the case when the antigenic substance is characteristic of a disease-causing agent. However, in some cases (particularly but not exclusively those cases where it is desired to raise antibodies against non-disease-causing agents), the antigenic substance may comprise a target antigenic moiety conjugated to a carrier. The carrier will generally be selected so as not to be recognised as "self" by the animal to which the vaccine is to be administered. Suitable carriers include albumins including ovalbumin (not for poultry), bovine serum albumin (not for cattle), human serum albumin (not for humans) and other albumins. Alternatively, the carrier may be a different protein or other molecule. Examples of proteinaceous carriers other than albumin include keyhole limpet haemocyanin and beta-galactosidase, among others. It is not necessary for the carrier either to be a protein or even proteinaceous, but such carriers are preferred. Carriers may in general be available from Sigma, Pierce or Bio Rad, or any other convenient supplier.

The nature of the implant vaccine described here also lends itself to the use of several antigens either linked to the same or different carriers. Similarly, in cases where immunological problems such as antigen competition occur or when one antigen preparation inacivates another via mixing, the implant vaccine may be formulated so that different antigens are presented in distinct implants keeping individual antigens separate.

The target antigenic moiety may be conjugated to the carrier, when a carrier is used, by any convenient means. Suitable conjugators include glutaraldehyde, toluene diisocyanate, carbodiimide, or any other suitable conjugator, which may effect a linkage through a carboxyamino group. Such groups may be created by means of activated diacid, such as an acid dichloride or an acid anhydride. Disuccinimidyl compounds are particularly suitable, especially disuccinimidyl tartrate and disuccinimidyl suberate, both of which are available from Pierce, as are many of the other conjugators that are preferred for use in this invention. Other acceptable conjugators effect a linkage through thiol groups as disulphides or thioethers; suitable conjugators include SPDP and other aminodisulphydril cross-linkers and double agents such as MBS.

The amount of antigenic substance present in each vaccine dose will of course depend on the identity of the antigenic substance and whether it is conjugated with a carrier. Typically, for a conjugate vaccine it may be expected that the amount of material administered per injection should be from 10μg to 10mg. For example in a GnRH vaccine, 2mg of conjugates may be present of which 100 to 800μg would be GnRH (typically 200μg of GnRH) and 1.9 to 1.2mg would be carrier. These amounts are purely illustrative and indicate suitable levels for GnRH vaccines.

The saponin may be obtained from any convenient source. Saponin is available from Sigma Chemical Co, USA, and a particularly purified and lyophilised form is available

from Superfos Biosector A/S, Denmark, under the trade mark QUIL-A. It should be noted that it is not a prerequsite that a single species be used; mixtures of different saponins are quite acceptable.Preferred saponins include those disclosed in WO-A-8809336.

The amount of saponin present can be any appropriate amount. Amounts of from 50μg to 50mg may be suitable, for example, from 500μg to 5mg; an amount of about 1mg may be found to be particularly appropriate.

The polycationic adjuvant may be any suitable such adjuvant, particularly including those disclosed in WO-A-8801177. Diethylaminoethyl dextran (DEAE-dextran) is particularly useful and may be supplied as the free base or the hydrochloride or any other appropriate acid addition salt. Other suitable polycationic adjuvants include polylysine, polyethyleneimine and chitosan, which again may be supplied either as the free base or as an acid addition salt. The polycationic adjuvant may be buffered to be at or near physiological pH, as will subsequently be described.

It should be noted that the invention contemplates the use of a conjugate of the antigenic substance and polycationic adjuvant as well as mere mixtures of two separate components. The antigenic moiety and polycationic moiety may therefore be covalently attached, either directly or by means of a linking element.

A vaccine in accordance with the invention can optionally contain certain other components. In

particular, the vaccine may contain a filler. The most preferred filler is calcium phosphate, particularly dibasic calcium phosphate dihydrate. A particularly suitable form of dibasic calcium phosphate dihydrate is sold under the trade mark EMCOMPRESS by Edward Mendell Co. Inc., Carmel, New York, USA. This preparation conforms to USP XX/FCC III. The average particle size of the calcium phosphate (or any other filler) may range from 20 to 200μm, with 50 to 150μm being a typical range. Average particle sizes of about 100μm are common. Alternative fillers may also be in the form of biodegradable polymers (see later).

The amount of calcium phosphate or equivalent filler may be such as to adjust the volume of the vaccine composition to a convenient amount. For example, a convenient maximum volume might be 1ml, but the circumstances will vary from case to case. The amount of calcium phosphate (or total filler) per unit dose vaccine formulation may range from 10mg to 1g, with from 20mg to 200mg being typical. The filler may comprise from 5 to 95% w/w of the weight of the formulation, with from 30 to 80% w/w being typical.

A further filler, which may for example be used in conjunction with the preferred calcium phosphate described above, is lactose. A suitable source of anhydrous lactose is direct compression lactose, such as that sold under the trade mark DCLactose 21 by De Melkindustrie Veghel BV of Veghel, The Netherlands. This formulation of anhydrous lactose satisfies the requirements of USP XXI/NF XVI. The amount of lactose

present can vary from 0 to 15% w/w, for example from 5 to 10% w/w, based on the total weight of the vaccine formulation.

Another filler which may be used is cholesterol. A suitable source is the USP grade from Croda Inc, USA. The amount of cholesterol present may vary from 0 to 80% w/w, for example from 25 to 50% w/w, based on the total weight of the vaccine formulation.

Other (generally dry) fillers may be present, for *p+91Xexample, sodium calcium hypophosphate or dry (for example freeze dried) aluminium hydroxide may be used as a filler.

Because preferred formulations of vaccines in accordance with the invention include tablets and extrusions, the presence of a lubricant to aid in formulation is desirable. Any suitable lubricant, such as magnesium stearate, can be used, but it is generally preferred for the lubricant to comprise a hydrogenated vegetable oil, such as that sold under the trade mark LUBRITAB by Edward Mendell Co, Inc, Carmel, New York, USA.

The lubricant may be present in an amount up to 5% w/w, based on the total weight of the vaccine formulation, but is generally present in a range of from 0.5 to 2.5% w/w.

Other adjuvants or components which stimulate the immune response may be present in vaccine formulations in accordance with the invention, if desired. For

example, muramyl dipeptide may be present. Lipid-based products may also be present for this purpose.

A buffer may be present, for example to counteract the effect that the polycationic adjuvant has on the pH when the vaccine is administered.

Other acceptable excipients can be present in the vaccine formulation in suitable amounts. It is however, not necessary for any other ingredients to be present.

The vaccines in accordance with the invention are solid and may therefore be in the form of a powder or granules, either of which may optionally be encapsulated, or compressed or otherwise prepared to form a tablet, bolus or extruded strip which may be cut or otherwise post-formed to any convenient length and/or shape.

In view of the generally solid nature of vaccines in accordance with the invention, they will generally be dry. This is not to mean that the vaccine as a whole, or any of the ingredients, is necessarily anhydrous.

Vaccines in accordance with the invention may be implantable and/or injectable, and will therefore for preference be sterile. A subcutaneously implantable vaccine is preferred, but an intramuscularly implantable vaccine is also viable. Intraperitoneally implantable vaccines are less preferred but may be suitable in some circumstances. It will not generally be appropriate to implant or inject vaccines in

accordance with the invention intravenously, as saponins have a powerful lytic effect on red blood cells.

Although there may be some applications in which the present invention is suitable for treating humans, species of animals which can usefully be treated by means of the present invention include cattle, pigs, sheep, deer, camels, horses, dogs and cats, to give but a few examples. In each of these and other species the vaccines of the invention can be used for conventional purposes for the treatment of disease. In addition, in each of these and other species, vaccines in accordance with the invention can be used for purposes other than preventing disease, for example for modulating hormone activity, particularly fertility hormone activity. In cattle, vaccines in accordance with the invention may be used bio-chemically to immunologically neuter bulls and cows. Immunoneutering of sheep and pigs is also a particularly preferred application. Immunocastration of ram lambs destined for the prime lamb market is a specific example.

It is by no means necessary for vaccines in accordance with the invention to be restricted to having a single function. Disease-preventing vaccines may be multifunctional, as may hormone activity-modulating vaccines. Additionally, vaccines in accordance with the invention can combine very different activities, such as disease prevention and hormone activity regulation.

Vaccines in accordance with the invention can be prepared by any convenient method, all of which are within the scope of the invention. It may be appropriate under some circumstances to prepare vaccines merely by adequately admixing the ingredients. According to a second aspect of the invention, therefore, there is provided a process for the preparation of a vaccine, the process comprising admixing (a) an antigenic substance capable of inducing the generation of antibodies on parenteral administration to an animal, (b) a saponin and (c) a polycationic adjuvant.

A particularly preferred way to prepare a vaccine in accordance with the first aspect of the invention involves freeze drying the components from a (for example aqueous) solution. For some reason that is not entirely clear, but may be to do with the degree of intimate admixture obtainable by such a process, vaccines prepared in this method have been found to be very satisfactory.

According to a third aspect of the present invention, therefore, there is provided a process for the preparation of a vaccine, the process comprising lyophilising a solution (for example an aqueous solution) of (a) an antigenic substance capable of inducing the generation of antibodies on parenteral administration to an animal, (b) a saponin and (c) a polycationic adjuvant.

The solution is preferably stirred thoroughly (for example, for at least 2 hours or even 24 hours or more) prior to lyophilisation for optimum results.

The solution will generally be aqueous and may include a buffer to bring the pH of the solution near to neutrality and/or physiological pH.

In certain cases (for example to prolong the release of active vaccine constituent) it may be preferred to admix the antigenic substance and the two adjuvants with the fillers by wet granulation and lyophilise the common mixture.

Although under some circumstances, as discussed above, the antigenic substance and the two adjuvants (the saponin and the polycationic adjuvant) can be lyophilised from a common solution, it may under some circumstances be possible to prepare satisfactorily an immunoadjuvant composition, to which the antigenic substance can subsequently be added.

According to a fourth aspect of the present invention, therefore, there is provided an immunoadjuvant comprising a saponin and a polycationic adjuvant.

As discussed above, vaccines in accordance with the invention are preferably solid. The vaccine may for preference be in tablet form or be formed by extrusion to a desired length. A vaccine including its active components in accordance with the invention may be coated. The coat may be water impermeable but erodible, so that after a suitable period of time the

coat will dissolve or otherwise break down to enable release of the active components of the vaccine. It is possible in this way to provide a plurality of implants, ranging from being non-coated to each having a coat of particular thickness and/or erodibility characteristics such that, for example, one implant might release active components immediately to provide a primary sensitising dose while others may release weeks or even months later to provide boosting doses and thereby extend the longevity of the immune response.

A variety of materials can be used for the coat, whether as an erodible or biodegradable coat. Polyesters constitute a preferred category of erodible/biodegradable encapsulating polymers that are also biocompatible; examples include polylactide, polyglycolide and poly(lactide-co-glycolide) such as those sold under the trade mark MEDISORB by the Dupont Company, USA., poly(hydroxybutyric acid) such as that sold by Chemie Holding, Linz, Austria, poly(hydroxybutyric acid-co-valeric acid) such as that sold by Aldrich Chemicals, USA, or ICI, UK. Other suitable erodible biodegradable polymers include polyacetals, polyorthoesters and polyorthocarbonates as is disclosed in EP-A-0052510 (Syntex). It will be appreciated that coatings can conveniently be made from a mixture of the above or other polymers, particularly when ester derivatives are used.

The coat may alternatively remain essentially intact after implantation; it may be semi-permeable to ensure adequate leaching out of ingredient. The coat may be

non-biodegradable if desired. Cellulose derivatives constitute a suitable category of polymer; examples include ethyl cellulose, such as that sold under the trade mark ETHOCELL by Dow Chemical Co, USA, methyl cellulose, such as that sold under the trade mark METHOCELL by Dow Chemical Co, USA and hydroxypropylmethyl cellulose, such as that sold under the trade mark PHARMACOAT by Shinetsu Chemical Co of Japan. Methacrylate derivatives form another suitable class. Examples include a 1:2 poly (methacrylic acid, methylmethacrylate) polymer sold under the trade mark EUDRAGIT S100 by Rohm Pharma, West Germany and 1:2:1 poly (butylmethacrylate, methacrylate, methylmethacrylate) polymer sold under the trade mark EUDRAGIT E100 also by Rohm Pharma.

It should be noted that the invention in certain circumstances (for example to allow enable pulsed antigen/adjuvant release at delayed time intervals) contemplates coating granules of the active antigen/adjuvant mix itself by solvent evaporation onto granules, wet granulation or fluidised bed spray coating or other means, with a mixture of the above or other erodible or biodegradable polymers prior to formulating into a vaccine as granulates or as compressed tablets. Such polymer coated granules are particularly useful as vaccine implants when used in conjunction with cholesterol as a filler.

According to a fifth aspect of the invention, there is provided a method of treating a human or another animal, the method comprising administering a vaccine in accordance with the first aspect of the invention.

The invention therefore encompasses the use of (a) an antigenic substance capable of inducing the generation of antibodies on parenteral administration to an animal, (b) a saponin and (c) a polycationic adjuvant in the preparation of a vaccine.

As vaccines in accordance with the first aspect of the invention can be used as one-shot vaccines, a single shot constitutes the preferred treatment regimen. However, the use of two- and multiple-shots is not ruled out, if the circumstances (or preference) require. If more than one administration is required, the time between administrations is preferably such as to give rise to an effective anamnestic response.

The invention will now be illustrated by the following examples.

EXAMPLE 1

The following examples illustrate the preparation of an antigenic peptide-protein conjugate in particular a GnRH based product for fertility control.

A Preparation of Antigen (Peptide-Protein Conjugate)

1g of GnRH modified at its carboxyl terminus from -gly amide to a -gly acid is added to 1g of ovalbumin in water. This is followed by the addition of a 25-fold molar excess over the peptide of 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide hydrochloride, giving a 0.25M

solution. The pH of the mixture is controlled at between 6.5 and 7 by titration with 1M hydrochloric acid for at least 5 hours, followed by dialysis against water and then reaction in 0.5M hydroxylamine at pH 7 for 5 hours. The final reaction mix is dialysed against water, filtered through a 0.2 micron membrane and freeze dried. Progress of the reaction to form peptide-protein conjugate, and dialysis to remove unconjugated low molecular weight by-products is monitored by analytical HPLC. The peptide content of the conjugate is determined by differential amino acid analysis relative to the amino acid content of carrier protein alone. (The treatment with hydroxylamine helps obtain a water-soluble product with consistent peptide content.)

B Preparation of Adjuvant

30g of DEAE-dextran (eg from Pharmacia, Sweden, or Sigma Chemical Co, USA) is mixed with 4.2g of saponin (eg from Sigma Chemical Co, USA or as a lyophilised preparation such as that sold under the trade mark QUIL-A from Superfos Biosector A/S, Denmark) and 2g of solid tris-(hydroxymethyl)aminomethane (eg Trizma Base Sigma Chemical Co, USA). The mixture is dissolved in distilled water (1.75 litres) and adjusted to pH 7 ± 0.2 units with a 2M aqueous solution of Trizma (pH 10.5).

C Preparation of Antigen-Adjuvant Mixture

Antigen peptide-protein conjugate prepared as described above, is then added to the neutralised adjuvant solution and dissolved by gentle mixing at ambient temperature (20°C). The solution is stirred thoroughly for at least 24 hours, prior to freeze drying. The dried antigen-adjuvant mix is passed through a stainless steel sieve (350μm mesh) prior to tablet preparation.

EXAMPLE 2

Tablet Preparation

A formulation to make a 100g powdered mixture for compressing into tablets (implants) is as follows:

	100g Batch	mg/tablet (average)
EMCOMPRESS Calcium phosphate	72.5g	170
DC-Lactose	8.0g	19
LUBRITAB Hydrogenated vegetable oil	2.5g	6
Antigen/Adjuvant mix from Example 1	17.0g	40
TOTAL WEIGHT :	100.0g	235mg

The batch is prepared by mixing the calcium phosphate and the lactose together in a tumble mixer at 27rpm for 15 minutes. The antigen/adjuvant mix from Example 1 is

then added, and the mixture is blended together for a further 15 minutes in an ERWEKA AR400 (trade mark) cube mixer from Erweka Apparatebau GmbH, Heusenstama, West Germany. The resulting mixture was sieved through a 350μm mesh, and the hydrogenated vegetable oil was added to the sieved mixture and then blended for 15 minutes, again in the ERWEKA AR400 cube mixer.

The blended mixture of ingredients is compressed into tablets in a 4.5mm punch and dye, using the MANESTY SP1 (trade mark) single punch tabletting machine from Manesty Machines Ltd, Liverpool, UK. The resulting tablets weighed 235mg ± 23mg, had a diameter of 4.5mm and a length of 8.6 ± 0.6mm.

EXAMPLE 3

The procedure of Example 1 was followed, except that the proportions of the adjuvants, buffer and antigenic conjugate were as follows:

Conjugate (GnRH-ovalbumin)	200mg
DEAE-dextran	6.0g
Trizma	400mg
Saponin	840mg

The DEAE-dextran, Trizma and Saponin were made up in 350ml distilled water and adjusted to pH 7 with 2M Trizma. A conjugate was then added to this solution, which was thoroughly mixed for 24 hours and then freeze dried. The resulting antigen/adjuvant mix was sieved (350μm mesh), then mixed with the other components in the amounts given below to form implants:

EMCOMPRESS Calcium Phosphate	30.31g
DC-Lactose	3.37g
LUBRITAB hydrogenated vegetable oil	1.04g
Antigen/Adjuvant Mix	6.88g
TOTAL WEIGHT :	41.6g

This mixture yielded up to 175 implants weighing approximately 235mg each. Each implant contained approximately 1.1mg of conjugate, equivalent to about 125μg GnRH.

EXAMPLE 4

The tablets produced in Example 3 were used to immunologically castrate rams (Dorset/Merino) as follows.

The rams were divided into six groups, each of five animals, and dosed with 1, 2 or 3 tablets in one or two implantations by subcutaneous implantation by means of a trocar in the neck region below the ear.

Testicular weight at various time intervals from the first implantation was measured by orchidometry, a comparative palpation procedure using a graded set of beads for reference. [C.M. Oldham et al Aust. J. Agric. Res. 29, 173-179 (1978)]. The second implantation was 4 weeks after the primary implant. The results eight weeks after the first implantation are shown in Figure 1 and demonstrate the ability of the implant

formulation to effect testicular atrophy in mature rams.

Example 5

The implant vaccines were used to examine the effect of changes in immuno-adjuvant formulation on testicular development in growing ram lambs. Groups of 5 second cross ram lambs 5 to 7 weeks of age were immunised subcutaneously in the neck below the ear with various GnRH vaccine implants having varying amounts and treatments of adjuvants. The implants were made as described in Example 3 except that the amounts of DEAE-dextran and/or Saponin were reduced. The amounts of Emcompress calcium phosphate were increased accordingly to maintain implant weights at approximately 235mg. The adjuvants, buffer and antigen conjugates were mixed in aqueous solution for 24 hours prior to freeze drying and incorporation into implants. One implant was given at primary (1^{o}) and one at the secondary (2^{o}) boost 5 weeks later. The results shown in Table 1 illustrate the effect of varying adjuvant formulation on testicular development in prepubertal ram lambs. Also shown is a dry mixed antigen/adjuvant formulation and a reference oil adjuvant vaccine [Hoskinson et al. Aust. J. BIOTECH 4, 166-170 (1990)] at 1mg antigen/2ml dose.

Table 1

Effect of Adjuvant formulation on testicular development in ram lambs.

Group Mean Testicular weight (g).

GROUP	WEEK: 0 (1°)	5 (2°)	9	13	22	Antibody titre at week 7 (1/5000cpm)
1. D1:S1 (STD)	10	25	16	17	111	7,666
2. D1:S1 (DRY MIX)	10	68	66	102	N.T.*	6,016
3. DO.5:S1	10	57	60	77	N.T.	7,099
4. DO.25:S1	10	55	68	122	N.T.	5,013
5. DO.S1	10	78	106	157	N.T.	4,580
6. D1:SO	10	51	83	124	N.T.	4,055
7. DO:SO	10	100	147	224	N.T.	411
8. D1:Q	10	24	26	32	74	10,320
9. VAX	10	25	34	20	78	10,523
10. CONTROLS	10	108	164	249	>280	29

CODE: D1, S1: DEAE-dextran and Saponin are in the same amounts as in Example 3.
DO, SO denotes the absence of DEAE-dextran or Saponin.
STD denotes standard formulation as in Example 3.

DRY MIX denotes antigen/adjuvant formulation dry mixed only before implant production.
DO.5, DO.25: DEAE-dextran at one half and one quarter respectively the amount in Example 3.
Q is Quil A Saponin at half the amount of Sigma Saponin in Example 3 and each implant has 2 mg antigenic conjugate instead of 1.1 mg.
VAX is the reference oil adjuvanted vaccine.
CONTROLS are placebo implants which contain carbodiimide treated ovalbumin instead of GnRH-ovalbumin conjugate.
N.T. denotes not tested.

Ram lambs are considered sexually competent when testicular weight exceeds 120 grams (WO-A-8801177). Table 1 shows that DEAE-dextran and Saponin alone or in combination retard testicular development in lambs when given as adjuvants in GnRH implant vaccines. Combinations of the two adjuvants have a more profound effect. Admixing the adjuvants and antigens in aqueous solution and lyophilising the mixture results in a more effective implant than simple dry admixing (compare groups 1 and 2). The results demonstrate the viability of solid implant vaccines in immunologically delaying puberty (compare groups 1 and 8 with 10). The formulation used gives comparable results to a commercial oil-based liquid vaccine (compare groups 1 and 8 with 9).

Example 6

The effect of implant GnRH vaccines (single administration) on testicular status in growing ram

lambs or mature rams were examined (Table 2 and Figure 2).

Groups of second cross ram lambs (3 to 5 weeks of age) and mature rams (12 months) were immunised subcutaneously by trocar in the neck region below the ear with GnRH vaccine implants. The implants were prepared as indicated for Group 8 in Example 5 (Table 1) in which Quil A saponin was used and each implant (235mg size) contained 2 mg of GnRH conjugate. The implants were used uncoated or were coated (10μm thick) with an under layer of hydroxypropylmethylcellulose ("Pharmacoat" HPMC 615;Shinetsu Chemical Co Ltd. Japan) to prepare a suitable surface for the main coat (80μm thick) of "Medisorb" 100DL lactide polymer (80-110k Daltons) applied in acetone: isopropanol (70:30 w/w) solvent. A protecting coat of HPMC 615 (10μm thick) was finally applied.

The implants were pan coated using an Erweka AR 400 drive unit, a 9.5 litre (type DK) coating pan and an Aeromatic (type Strea-1) spraying device with ER 39 nozzle (1.1 mm orifice).

Table 2

Group mean testicular weight (g).

Group A	Week 0	5	7	10	15
Ram Lambs (n=7)					
1. Q I (1^{o} only)	14	12	19	41	80
2. Coated QI (1^{o} only)	10	19	30	65	121
3. QI + coated QI (1^{o} only)	10	14	21	31	61
4. QI (1^{o} then 2^{o} at week 5)	10	14	14	16	19
5. VAX (1^{o} then 2^{o} at week 5)	10	13	11	16	10
6. Controls	10	28	38	78	118

GROUP B	Week 0	4	8	12	16
Mature Rams (n=8)					
1. QI (1^{o} only)	234	208	138	144	162
2. Controls	244	220	222	209	210

CODE QI denotes an implant prepared with Quil A Saponin and 2mg antigen conjugate as in Example 5, Table 1 Group 8.
Coated QI denotes that the implant was subsequently coated as described in the text.
VAX is the reference oil adjuvant vaccine.

Controls are placebo implants as described in Table 2.

The results demonstrate that a single implantation in either immature or mature rams will suppress or regress testicular development. Whilst a secondary boost enhances the effect, a coated implant given at the same time as the first implantation allows for implants with a delayed release (compare Groups A 3 and A 4).

In another group of ram lambs an uncoated implant prepared according to Example 3 was given to each lamb in conjunction with an implant that contained cholesterol filler in various amounts in place of calcium phosphate. The results are shown in Figure 2 and demonstrate that the use of cholesterol as an additional filler (between 20% and 80% of implant weight) can be used to advantage in constructing solid vaccines suitable for single implantations.

EXAMPLE 7

In order to demonstrate the solid implant vaccine approach for disease applications in animals we undertook experiments to test serological responses to a number of relevant antigens. In each case the antigens were produced by Arthur Webster Pty. Ltd. (an Australian veterinary vaccine manufacturer) of Sydney, Australia. The example shown is a solid implant vaccine for ovine footrot and is preared from concentrated purified Bacteroides nodosus pilus antigens derived from recombinant Pseudomanas aeruginosa representing the nine B. nodosus serogroups

A to I. All antigens were mixed together before blending into vaccine. The aqueous solution of antigen representing 100 doses was freeze dried. The dried mixture was then formulated with the following components in a manner similar to that described for Example 3.

DEAE-dextran	3.4g
Trizma	230mg
Saponin	480mg
Dried Antigen mix	100 doses
Water	200ml

The mixture was carefully stirred to dissolve the components and the pH was adjusted to 7.0 with 2M Trizma. The solution was stirred for 24 hours at 20°C prior to freeze drying. The dried antigen/adjuvant mix was sieved through a 350μm stainless steel mesh.

Formulations were made to contain the equivalent of either one dose (A) or about half dose (B) of antigen per implant as follows:

	A	B
EMCOMPRESS Calcium Phosphate	8.7g	9.6g
DC-Lactose	0.97g	1.07g
Lubritab	0.3g	0.3g
Antigen/Adjuvant	2.0g	1.0g

Implants were made as described in Examples 2 and 3 and administered via trocar. A single implant was used at each vaccination except where designated as "A+B" in Table 3 below - in these cases the animals were vaccinated both with one A and with one B tablet at the

same time at the same site. An oil adjuvanted liquid vaccine in 1ml volume served as a reference standard - this was prepared from the same antigen mix at the dose level of the A implants.

Groups of 8 sheep were immunised with a 4 week interdose interval. To illustrate the immune response, individual sera were tested for response to each of 5 serogroups (A,B,C,D, and I); results presented below (Table 3) are grand geometric means (GGM) i.e. the mean of the geometric means for the 5 serogroups. The sera from the sheep were tested at various intervals during the trial using a normal microtitre plate agglutination assay.

Table 3

Antibody titrations for footrot vaccines

GMM at various time intervals

VACCINE GROUP	WEEK 0(1^{o})	4(2^{o})	7	11
A(1^{o})/A(2^{o})	NT	760	4020	1440
A(1^{o})/B(2^{o})	NT	830	4160	1350
(A + B) 1^{o} only	NT	760	790	NT
Standard 1^{o}, 2^{o}	NT	250	1330	770
Controls	60	70	70	NT

The following codes designate the vaccine treatment:

A(1^{o}), A(2^{o}):	Implant A at first dose /Implant A at boost.
A(1^{o}), B(2^{o}):	Implant A at first dose /Implant B at boost.
(A + B) 1^{o} only:	Two implants A and B at first dose, no boost dose.
Standard 1^{o}, 2^{o}:	Conventional oil vaccine at first dose. Conventional oil vaccine at boost.
Controls:	Unvaccinated sheep.
N.T.:	Denotes not tested

The results clearly show the solid implant formulations stimulate relatively higher levels of antibody production than the reference oil adjuvanted vaccine, provided that a second dose (boost) is given. These results are particularly significant in that the implants provide suitable levels of antibody in a regimen commensurate with current farm management practices. Implants coated with different thicknesses of polymer would provide the basis of booster effects from a single implantation strategy.

Similar positive results for the solid implant vaccine approach were obtained with Caseous lymphadenitis

antigen in sheep, Botulinum in cattle and Bovine Ephemeral Fever, when compared with the conventional liquid vaccines currently used for these diseases.

In all implantations, whether for hormone or disease vaccine, the site reactions were trivial and/or non-existent and by two weeks post vaccination had disappeared. In particular the presence of cholesterol in formulated implants has the added advantage of reducing the toxicity of the saponin and may thus decrease the site reaction further.

CLAIMS

1. A solid vaccine composition comprising an antigenic substance capable of inducing the generation of antibodies on parenteral administration to an animal, a saponin and a polycationic adjuvant.

2. A vaccine according to Claim 1 wherein the antigenic substance gives rise to antibodies against a disease causing agent.

3. A vaccine according to Claim 2 wherein the disease causing agent comprises bacteria, virus, fungus or protozoa.

4. A vaccine according to Claim 3 wherein the disease causing agent comprises the bacteria causing foot rot, botulism or caseous lymphadenitis (CLA) or the viruses causing bovine ephemeral fever (BEF) or foot and mouth disease.

5. A vaccine according to Claim 1 wherein the antigenic substance gives rise to antibodies against an agent which does not normally cause disease.

6. A vaccine according to Claim 5 wherein the agent is a peptide or a non-peptide hormone.

7. A vaccine according to Claim 6 wherein the agent is gonadotrophin releasing hormone (GnRH).

8. A vaccine according to Claim 6 wherein the agent is growth hormone.

9. A vaccine according to claim 1 wherein the antigenic substance comprises the entity against which antibodies are to be raised.

10. A vaccine according to claim 1 wherein the antigenic substance comprises a target antigenic moiety conjugated to an immunogenic carrier.

11. A vaccine according to Claim 10 wherein the carrier is a proteinaceous material.

12. A vaccine according to claim 1, additionally including a filler.

13. A vaccine according to Claim 12 wherein the filler comprises calcium phosphate.

14. A vaccine according to Claim 12 wherein the filler comprises cholesterol.

15. A vaccine according to claim 1 which is formulated as a powder, granules, tablets, boluses or extruded strips.

16. A vaccine according to claim 15 which is adapted to be implanted into a patient.

17. A vaccine according to claim 1 for fertility control and immunoneutering of animals.

18. A vaccine composition acccording to claim 15 which is coated with a polymer which is water impermeable but erodible or is semi-permeable.

19. A vaccine composition according to claim 18 containing a plurality of implants, the implants having coats of various thicknesses and/or erodibility characteristics such that periodic delivery of the antigen/adjuvant doses can be achieved.

20. An immunoadjuvant comprising a saponin and a polycationic adjuvant.

21. A vaccine according to claim 1 or an immunoadjuvant according to claim 20 wherein the polycationic adjuvant comprises diethylaminoethyl dextran (DEAE-dextran) or a salt thereof.

22. The preparation of a vaccine according to claim 1 by the admixing of:

(a) an antigenic substance;
(b) a saponin; and
(c) a polycationic adjuvant.

23. The preparation of a vaccine according to claim 22 comprising lyophilising a solution of:

(a) an antigenic substance;
(b) a saponin; and
(c) a polycationic adjuvant.

24. The preparation of a vaccine according to claim 23 wherein the solution is an aqueous solution.

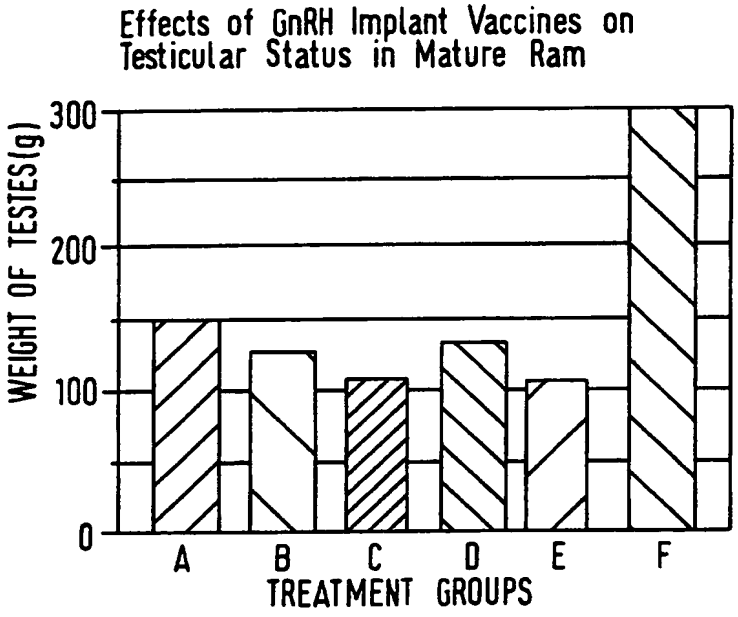
25. The preparation of a vaccine according to claim 22 wherein an antigenic substance, a saponin and a polycationic adjuvant are admixed by wet granulation optionally in the presence of a filler, and the common mixture is lyophilised.

26. The preparation of a vaccine according to claim 1 comprising coating granules of the active antigen/adjuvant mix by solvent evaporation on to the granules, wet granulation, or fluidised spray coating or other means, with a polymer or a soluble mixture of polymers, followed by the formulation into a vaccine as a granulate or compressed tablets.

27. A method of treating an animal by means of administering a vaccine according to claim 1.

28. The use of an antigenic substance capable of inducing the generation of antibodies on parenteral administration to an animal, a saponin and a polycationic adjuvant in the preparation of a solid vaccine composition.

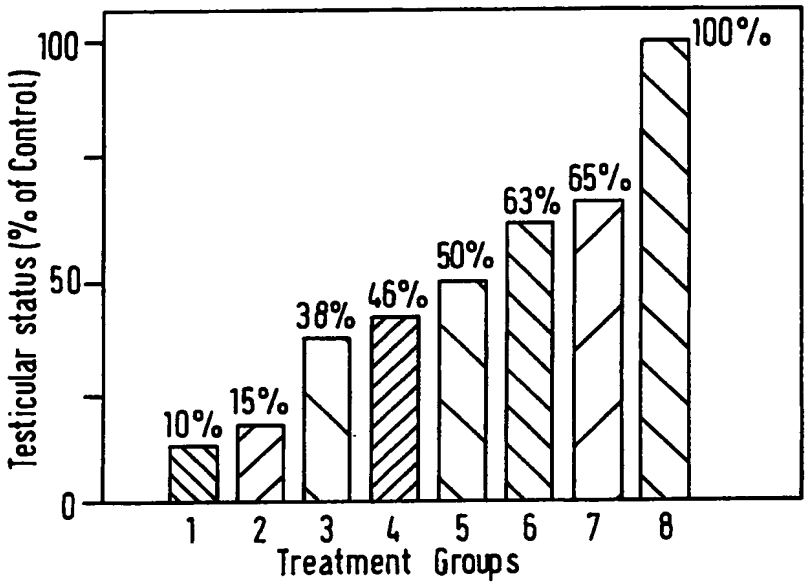
FIG.1



Group A 1 implant on 1 occasion
Group B 1 implant on 2 occasions
Group C 2 implants on 1 occasion
Group D 2 implants on 2 occasions
Group E 3 implants on 1 occasion
Group F Controls

FIG.2

Effects of cholesterol filler in GnRH Implant Vaccines on Testicular Status in Growing Ram Lambs



1. Reference of 1 adjuvant Vaccine 1° followed by 2° 4 weeks later
2. D1:S1 implant vaccine 1° followed by 2° 4 weeks later
3. D1:S1 Plus D1:S1 with 50% cholesterol filler; 1° only
4. D1:S1 Plus D1:S1 with 80% cholesterol filler; 1° only
5. D1:S1 Plus D1:S1 with 20% cholesterol filler; 1° only
6. D1:S1 Plus D1:S1 with 10% cholesterol filler; 1° only
7. D1:S1 Plus D1:S1 with no cholesterol; 1° only
8. Controls (1° only); Mean Testicular weight at week 8 is 135g

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 90/01459

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[5]: A 61 K 39/39, 39/00, 9/14, 9/20

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[5]	A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X	EP, A, 0284406 (COOPERS ANIMAL HEALTH LTD) 28 September 1988 see page 6, lines 30-37 (cited in the application)	20
A	WO, A, 87/06129 (DARATECH PTY. LTD) 22 October 1987 see the claims (cited in the application)	1-26,28

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
27th November 1990	18. 12. 90
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	M. PEIS

Form PCT/ISA/210 (second sheet) (January 1985)

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. [X] OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This International search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. [X] Claim numbers 27, because they relate to subject matter not required to be searched by this Authority, namely:

Pls. see Rule 39.1(iv) - PCT:

Method for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods.

2. [ ] Claim numbers....., because they relate to parts of the International application that do not comply with the prescribed requirements to such an extent that no meaningful International search can be carried out, specifically:

3. [ ] Claim numbers....., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. [ ] OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this International application as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International search report covers all searchable claims of the International application.

2. [ ] As only some of the required additional search fees were timely paid by the applicant, this International search report covers only those claims of the International application for which fees were paid, specifically claims:

3. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. [ ] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

[ ] The additional search fees were accompanied by applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. GB 9001459 SA 40378

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 07/12/90
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A- 0284406	28-09-88	AU-A-	1496888	02-11-88
		WO-A-	8807547	06-10-88
		JP-T-	1502753	21-09-89
WO-A- 8706129	22-10-87	AU-A-	7237087	09-11-87
		EP-A-	0265457	04-05-88
		JP-T-	1500034	12-01-89